9.—COPPER BUNTICIDES.

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INTRODUCTION.

In 1925 the Department of Agriculture purchased commercial "copper carbonate" from six different firms in Perth and submitted samples to the Government Chemical Laboratory with a view to determining the best dust based on the suggested standards of Mackie and Briggs. While none of the samples conformed to the standards, the one nearest approaching them gave markedly inferior control of bunt infection in trial plots over several seasons.

The purpose of this investigation has been to determine—

(a) standards for copper dusting powders;

(b) chemical and physical characteristics of the powders;

(c) the action of the powders on bunt spores.

Commercial "copper carbonates" vary in composition and include basic carbonates, basic sulphates, mixtures of these compounds, and oxychlorides. Rarely, if ever, do the prepared compounds approach the constancy of composition of the naturally occurring minerals, their compositions depending entirely on the methods of preparation. Experience in Western Australia suggests that the basic sulphates and mixtures are better controllers of bunt than the basic carbonates, and again, for no apparent reason, some very fine-grained basic sulphates are inferior to coarse-grained ones. Beyond making comparative trials no special work has been done on the oxychlorides.

FIELD TRIALS.

The field trials have been carried out at the Merredin and Chapman experiment farms. Merredin soil may be described as a heavy chocolate loam with a pH value of 7.1. In 1928 experiments were duplicated on another portion of this farm in poor yellow-brown sand containing a small percentage of clay. This soil had a reaction of pH 5.8; it is poor wheat land but grows excellent rye. During the same year small trials were conducted at Apple-cross, near Perth, in grey sand devoid of clay, reaction pH 6.1 and containing 3 per cent. of organic material. Unfortunately the latter results can only serve as an indication, as a fire destroyed the crop before the harvest was completed.

During the 1925 trials it was shown that the powders almost completely controlled the disease when the rate of infection was one part of bunt spores to 750 parts by weight of wheat and applied at the rate of 2 ozs. per bushel.

In order to show greater differences in effectiveness of the powders the spore dosage was increased to 20 parts, and over later years dropped to 10 parts. A variety of wheat known as *Booran* was first used on account of its low bunt resistance, but was later changed to *Gluyas Early*.

All seeds were infected and treated in the laboratory and placed in test tubes plugged with cotton wool. It was noticed that the basic carbonates had a tendency to lift the spores when shaken in a stoppered bottle with the infected grains. One hundred infected and treated seeds were sown by hand in rows 2¼ links apart, each treatment being repeated five times and planned on the "chess-board" system, with appropriate controls. Details of the cultural methods adopted will be found in the "Journal of Agriculture, W.A." At harvest time the infected and clean plants were counted and the diseased plants expressed as a percentage of the matured ones.

PARTIAL CHEMICAL AND PHYSICAL COMPOSITION OF THE COPPER DUSTS.

Table 1 gives the partial chemical and physical composition of all dusting powders used during the tests :—

TABLE 1.

Sample.	Cu.	CO_2	Acid sol. SO_3	Water sol. SO_3	Combined Cl	Density lbs. per cub. ft.	Retained on 200 mesh sieve
	%	%	%	%	%		%
A 25	46·88	0·88	0·43	3·83	17·09	63·2	43·6
B 25	50·04	17·64	·49	2·54	...	58·5	0·6
C 25	51·08	11·94	6·41	0·98	...	61·0	17·5
D 25	49·88	2·00	17·03	0·72	...	76·9	5·15
E 25	47·76	0·56	19·92	1·61	...	68·1	7·2
F 25	53·48	6·64	12·11	0·82	...	72·2	8·3
A 27	50·4	2·20	...	...	...	89·3	...
B 27	51·44	17·96	...	...	...	53·8	...
A B	52·48	19·32	...	...	...	49·0	3·8
Basic sulphate	52·3	...	16·2	Trace	...	51·0	...
Copper hydroxide ...	63·0	...	...	...	...	...	...
Sm	54·36	...	...	...	15·08	40·2	12·2
Copper acetate	29·96	...	...	...	...	...	...

Uniformity in composition is only shown by the copper content, which averages about 50 per cent. The basic sulphate was prepared by adding recently ignited pure lime to a solution of pure copper sulphate and the calcium sulphate precipitated at the same time washed out with distilled water. Its composition was closely related to $CuSo_4.3Cu(OH)_2$. The copper hydroxide was prepared by adding ammonia to a boiling copper sulphate solution until most of the copper was thrown out as a green precipitate. The precipitate was filtered and washed, treated with strong caustic soda solution for one hour, and again washed by decantation and dried. The copper hydroxide retained its bright colour for several months before finally turning black. The specially prepared samples were ground to pass a 200-mesh sieve. Apparent densities were determined by tamping down on a table an ounce of material in a 50 ml. cylinder until the volume was constant. Density determinations will, however, be discussed later.

At the commencement of this investigation only some of the samples mentioned in Table 1 were used and gave the following results when tested in the field:—

TABLE 2.

	1925. Spore dosage 1–750 ; per cent. infected plants.		1926. Spore dosage 20–750 ; per cent. infected plants.	
—	Merredin.	Chapman.	Merredin.	Chapman.
A 25	...	...	34	5
B 25	...	...	44	5
C 25	1	...	48	6
D 25	...	...	24	3
E 25	...	...	49	15
F 25	1	...	31	5
Sm	...	...	40	13
Copper acetate	1	...	42	10
Average controls	22	13	76	83

Attempts which were made to correlate effectiveness of bunt control with the chemical and physical properties of the powders gave abortive results. Sample B25 is the only one approaching the standards recommended by Mackie & Briggs excluding of course A25, Sm and copper acetate, which are special cases. B25 closely resembles a sample of copper carbonate obtained from California and described as a satisfactory powder; unfortunately the latter sample was too small for use in field trials. D25, despite its high density, has proved its efficiency in several types of soils over many years. It is a mixture of basic sulphates and carbonates, while a complete chemical analysis shows its components are not combined in any simple ratio. The local agents for the material have not been able to trace the manufacturers. Apart from confirming these results several lines of attacking the problem presented themselves:—

A. Preparation of other copper dusts of known composition and further trials with additional commercial compounds.

B. Determinations of the amount of powder actually retained on the infected and clean wheat grains.

C. Investigating the relative solubility of the compounds in the soil solution surrounding the germinating wheat grains and spores.

D. Determination of specific chemical and physical characteristics.

PREPARATION OF OTHER COPPER DUSTS OF KNOWN COMPOSITION AND FURTHER TRIALS WITH ADDITIONAL COMMERCIAL COMPOUNDS.

A. For the 1927 trials the coarse oxychloride A25 was ground to pass a 200 mesh sieve to compare it with the original powder. Two new commercial samples were tried, B27 a commercially pure basic carbonate of lower density than B25 and manufactured by the same firm, and another A27 somewhat similar in composition to D25 but of higher density. In addition the basic sulphate previously described (page 86) was tested before washing out the associated gypsum. The results of field trials are given in Table 2.

TABLE 2.

	1927. Spore dosage 20–750; per cent. infected plants.	
	Merredin.	Chapman.
A 25 (ground)	75	7
A 25	77	8
B 25	77	7
C 25	69	6
D 25	63	6
E 25	70	7
F 25	60	7
A 27	61	4
B 27	77	7
Sm	70	5
Basic copper sulphate and gypsum	71	11
Average controls	...	60

Due to secondary causes many of the plants in the control rows failed to reach maturity. The season at Merredin was apparently favourable for the development of bunt as the control by the fungicides was markedly less than in preceding and succeeding years. The differences were small so it is hard to draw definite conclusions. Grinding sample A25 did not improve it. D25 was inferior to F25, and A27 at Merredin was excelled only by A27 at Chapman and equalled by C25. The basic copper sulphate and gypsum mixture was the worst at Chapman and a very poor one at Merredin.

In 1928 the field experiments were modified. It was decided to exclude Chapman farm and duplicate the trials on different soils at Merredin. Samples A25, C25, E25 and F25 were abandoned. Basic copper sulphate washed free of gypsum, a new basic carbonate AB of low density and mixtures of the basic sulphate with AB were included as well as copper hydroxide. The dosage was decreased to 10-750.

TABLE 3.

Spore dosage 10–750 ; per cent. infected plants.

	1928. Merredin Farm.	
	Loam soil.	Sandy soil.
B 25	33	3
D 25	12	Nil
A 27	26	3
B 27	51	7
AB	57	12
Basic copper sulphate	21	6
Mixture 1	29	4
„ 2	29	3
„ 3	27	3
„ 4	31	2
„ 5	31	4
Copper hydroxide	31	3
Sm	48	3
Average controls	83	91

The mixtures were prepared by mixing increasing proportions of AB with the basic sulphate in the ratios 1-11, 9, 7, 5 and 4. Mixture 3 had roughly a chemical composition similar to D25.

D25 again showed its ability to control the disease compared with the basic carbonates. All plants, both affected and clean, stooled remarkably well and made excellent growth in the loamy soil while growth generally was stunted and poor in the sand. It is somewhat strange the rate of infection should be much higher in vigorous plants that have a better chance of combating the disease than in the less vigorous ones. The explanation probably lies in the facts that the soil assists the fungicide in its action or the poor plant is an unsuitable host for the fungus.

The addition of basic carbonate to the basic sulphate reduced the fungicidal properties of the basic sulphate in the loam and increased them in the sandy soil. The results so far indicate that those samples which contain a high percentage of basic sulphate are superior to those in which basic carbonate predominates; unfortunately this statement is not absolute as powder E25 used in previous trials provides a notable exception. This powder is almost a pure basic sulphate and though very finely ground it has a high density.

It has been suggested that the copper which is linked to the hydroxyl group in the basic copper salts is the active agent of the fungicide, but the copper hydroxide used in 1928 contained 63 per cent. of copper and only gave average results in the field trials. Calculations have been made of the hydroxide content of many of the dusts used but no relationship exists between the percentage present and the fungicidal properties.

The figures for the Applecross trials are not given; the results indicated, however, that the controls were high while the fungus appeared easy to control. Sodium fluoride was used in one treatment; it did not appear to be effective, and at the same time retarded the germination and development of the plants.

THE AMOUNT OF DUST ACTUALLY RETAINED ON CLEAN AND INFECTED SEED.

Western Australia standard fair average quality wheat was dusted with several of the powders at the rate of 2 ozs. per bushel of wheat. The dusted sample after being thoroughly shaken in a stoppered jar was thrown on to a 30 mesh sieve and gently rolled over the surface to remove the unattached dust. After this it was found that the adhering dust could be removed by adding 2.5 grams of the wheat to 20 mls. of cold .2NHCl allowing the acid to react 4 minutes with constant stirring to dislodge air bubbles clinging to the grain. The mixture was filtered directly into 100 mls. Nessler tubes and washed to 75 mls. with cold distilled water. The copper in the filtrate was then determined by the ferrocyanide method. The acid treated wheat was carefully ashed and no copper was found in the residue.

Another portion of wheat was infected with bunt spores at the rate of 10 parts of spores to 750 parts of wheat by weight. The bunted wheat was then treated with the powders and the adhering dust determined in exactly the same manner as for the clean seed.

Table 4 gives the results of these experiments.

TABLE 4.

Sample.	B25.	D25.	E25.	AB.	Basic sulphate.
Clean Seed— Percentage of added dust adhering	72	60	76	72	76
Bunted Seed— Percentage of added dust adhering	68	88	80	46	84

It was observed that many spores were removed from the grains by AB, and less by B25.

At the heavy spore dosage the spores assist some of the powders to adhere to the grain while other powders remove spores and themselves in the process of treatment. These facts throw doubt on the reliability of field trials, as the actual infected and treated seed sown in the field is sometimes not what it is specified to be. Unless large quantities of material are available it is impossible to determine accurately the amounts of bunt spores dislodged by the powders. Mackie & Briggs have recorded experiments using basic copper carbonate at heavy spore dosages over two seasons. It is presumed the same powder was used. In 1921 and 22 at a spore dosage of 1-30 by weight they obtained the following results by infected heads count:—

	1921.	1922.
	Per cent. bunt.	
Copper carbonate 1 oz. to bushel	30	36.6
„ „ 2 „ „	17.7	46.5
„ „ 4 „ „	3.4	20.0

Their 1921 figures are what one might expect if the powder did not lift the spores. Analogy with sample AB suggests approximately 1 oz. of the carbonate would not dislodge many spores. The 1922 results suggest dislodgment of spores and dust, the dust deficiency being made up at the 4 ozs. rate with, in proportion, fewer spores dislodged compared with dust retained. Admittedly their results are hard to explain unless different powders were used for the different years.

The ratios of powders on bunted seed to powders on clean seed are interesting when compared with the relative efficiency of the powders over a number of years.

TABLE 5.

Sample.	B25.	D25.	E25.	AB.	BS.
Ratio $\left\{\dfrac{\text{powder on bunted seed}}{\text{powder on clean seed}}\right\}$ ...	·94	1·33	1·05	·64	1·11
Dosage—		Percentage Infected Plants.			
20–750 1926 Merredin loam	44	24	49	...	...
„ Chapman	5	3	15	...	...
20–750 1927 Merredin loam	77	63	70	...	...
„ Chapman	7	6	7	...	...
10–750 1928 Merredin loam	33	12	...	57	21
„ Merredin sand	3	*Nil*	...	12	6
10–750 1929 Merredin loam	12·4	4·4	...	...	2·2
10–750 1931 Merredin loam	*Nil*	0·9	...	...	0·7

Generally speaking the samples with the highest ratios are the better bunt controllers, the outstanding exception being E 25. A standard for fineness based on the amount of dust clinging to clean or infected seed does not seem feasible.

All workers on bunt control cannot have failed to notice the great differences in the counts of infected plants in different rows under the same treatment. Similar variations exist in infected and untreated rows, the following figures provide typical examples:—

Variety of Wheat.	Infection 10–750.	Percentage infection per row.					Average.
		Row 1.	Row 2.	Row 3.	Row 4.	Row 5.	
Booran	10–750	74	53	62	80	66	67
Gluyas Early	,,	39·5	40	42	45·25	41·25	41·6
Infected and treated Gluyas Early—							
Copper compound B25	,,	9	6	19	18	10	12·4
Basic sulphate ...	,,	2	1	1	2	5	2·2

Several factors could account for these variations:—

1. Variations in the soil—

    (a) texture,

    (b) moisture content,

    (c) temperature.

2. Distribution of spores over the surface of the grain.

3. Position of the grain in the seed bed.

4. Depth of planting.

5. Method of calculating percentage of infected plants.

1. The average control figures for Merredin loam and sand in 1928 were 83 and 91 per cent. infected plants respectively. The plots on which these experiments were carried out were about half a mile apart and were therefore under similar climatic conditions. Soil temperatures possibly varied. The Chapman soil is somewhat sandier than the Merredin loam, usually the infection is high at Merredin, the year 1926 being a notable exception when the figures were 76 and 83. Twentyman reports variations on different farms in Victoria. While infection varies from year to year in Western Australia the control of the disease by bunticides is easier in lighter soils.

A partially dry seed bed would probably germinate spores but not seed, the spores would develop and then dry off without the germ tubes penetrating the plumule.

The optimum germination temperatures of wheat and bunt differ, the latter being lower. If the wheat plant is germinating slowly due to cold the spore has a better chance of reaching the plumule before its cuticle hardens. A quick growing plant would have a better opportunity to escape infection. On the other hand if it is too cold for wheat germination, the spore will germinate and die before it can penetrate the seedling.

2. On shaking loose spores with wheat in a closed vessel it is noticed that each grain apparently picks up as many spores as its neighbour. The spores cling more readily to some parts of the grain, notably the brush, the remainder spread themselves more or less evenly over the entire surface. It is highly probable that those spores nearest the germ are in a better strategical position to deliver an attack with their germ tubes on the emerging plumule. Particularly would this be the case when conditions were unfavourable for the development of spores. An infected grain planted crease down, *i.e.*, germ up, is less likely to be attacked below the first joint of the young plumule by a germ tube travelling from the brush than from the immediate neighbourhood of the germ. Control experiments in which half the grains were planted crease up and half down were conducted at Applecross in sandy soil; 200 grains in all were sown. No differences were shown in the final count, but it must be admitted the conditions were ideal for spore germination. The counts in each row were equal.

3. By repeating experiments a number of times errors due to an irregular seed bed may be reduced to a minimum when computing a final result. Surface moisture content will vary considerably after rain, particularly on a heavy soil such as Merredin; it might be several days before the moisture content of the mulch is evenly distributed at the depth of planting. The irregular way in which the germinating seed lies combined with variations in the condition of the mulch, is suggested as a reason for fluctuations in the counts of infected plants in different rows. Experience in Western Australia shows infected plants to be grouped together, it being the exception to find an isolated bunted plant between healthy ones. Twentyman in Victoria has made a similar observation.

4. Twentyman has carried out experiments planting at different depths but was unable to draw any definite conclusions.
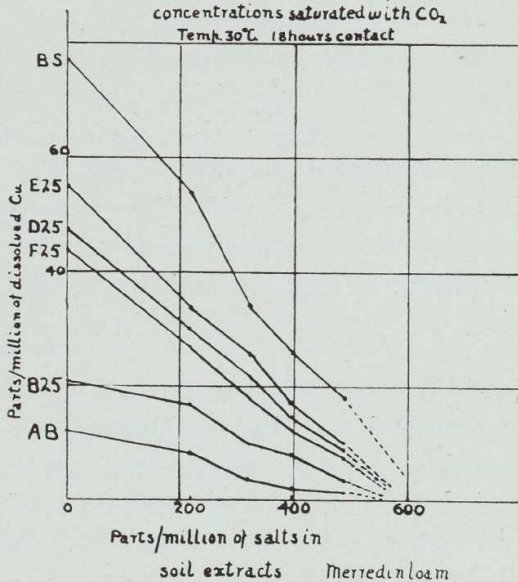
5. Some experimentalists prefer to count bunted heads rather than plants, the reason is difficult to understand. The main function of a bunticide is to prevent disease. There is no evidence that one spore cannot be responsible for infecting every head on a plant, so it seems a plant count gives a better idea of relative efficiency. Percentage infection has been determined by dividing the number of infected plants by the total number matured and multiplying by one hundred. In some cases plants have died after 30 days when final germination counts were taken. It is highly probable most of these were bunted and consequently less able to withstand attacks from other diseases. Were such an assumption correct then the percentages of bunted plants should be higher than reported. More plants die in the infected control rows than the treated ones.

C.—RELATIVE SOLUBILITY OF THE COMPOUNDS IN THE SOIL SOLUTION SURROUNDING THE GERMINATING WHEAT GRAINS AND SPORES.

The marked variations in the counts of the same treatments planted in different rows suggested attacking the problem from a chemical point of view. It was realised that the copper, to be effective, must be dissolved before it can penetrate the spore or its promycelium and germ tubes. The solubilities of the compounds were therefore determined in various solvents. Though insoluble in water the copper dusts were found to dissolve readily in some weak acids and alkalis.
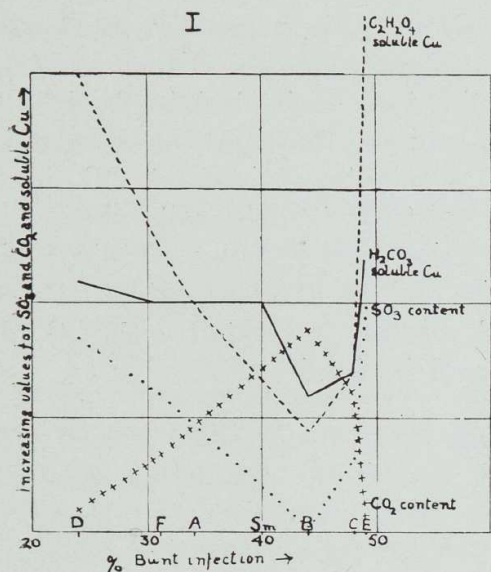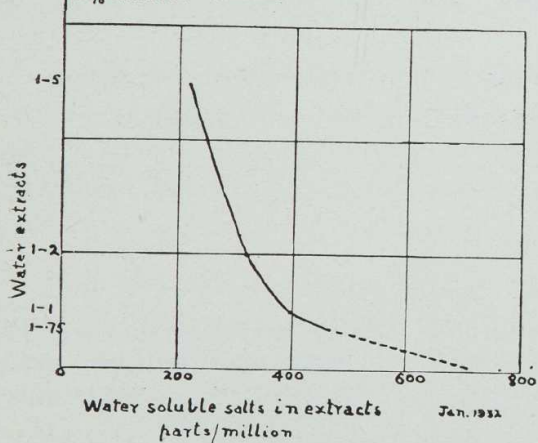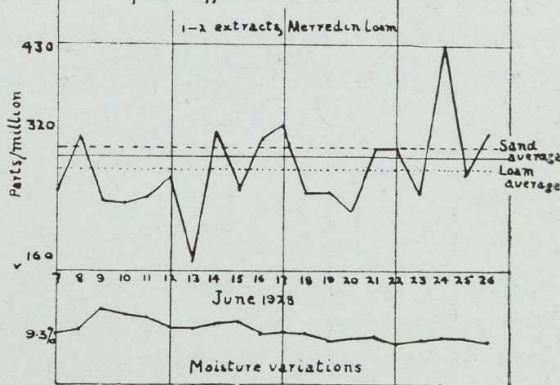
Solubilities in carbonic and oxalic acids increase and decrease depending on the amounts of sulphate and carbonate in the original material. The SO_3 curve in the accompanying graph, No. I., closely follows the H_2CO_3 soluble copper, when allowance is made for the oxychlorides which have been included in the solubility curves as a matter of interest. The graphs are purely relative ones, the determined radicles being plotted against the percentage of infection recorded in 1926 at Merredin. The Chapman figures do not lend themselves to graphing owing to the graphs coinciding in too many places, but the general forms are similar.

The graphs at first sight suggest that a fairly high solubility in H_2CO_3 is necessary for bunt control, but on the other hand too high a solubility is detrimental; this would explain the poor fungicidal properties of E25, but unfortunately a basic sulphate (B.S) was later prepared which had a greater solubility than E25 and was a better bunt controller. It has not yet been shown that during the control of the disease the spore is killed or its germination suppressed by the copper dust, or whether the growth from the spore is poisoned after germination. The solubility suggested that a moderately soluble compound would maintain a lethal dose of copper over a longer period than an insoluble or very soluble one. The soluble copper is precipitated by the soil solution or absorbed by the soil colloids almost as fast as it is dissolved.

To appreciate the H_2CO_3 solubility theory it is necessary to visualize what is taking place in the seed bed. A dry grain covered with an intimate mixture of bunt spores and copper dust lies in a moist bed in fairly close contact with soil particles. Soil moisture passes through the spores and dust into the grain which commences to germinate, giving out CO_2 in the process. After a period of time the soil moisture, *i.e.*, the soil solution immediately in contact with the grain and passing into it, must contain a high percentage of CO_2 derived from the grain and to a lesser extent the soil atmosphere. The possibility of this soil solution saturated with CO_2 dissolving the copper dust has been investigated in detail.

200 grains of *Booran* wheat weighing 9.33 grams were placed on a filter paper in a 200 cc. Erlenmeyer flask containing wet cotton wool and attached to a CO_2 absorption apparatus, using soda lime to absorb the liberated CO_2. The flask was covered with black paper, effectively blocking out the light. At the end of each 24-hours period 2 litres of CO_2 free air were drawn through the apparatus and the absorption tubes weighed. At the end of the experiment water was added to the flask and the contents boiled and 2 litres of air again passed. The following results were obtained :—

Date.	Time.	CO_2 liberated gms.	Temperature.		Remarks.
24–7–28	a.m. 11	...	12 p.m.	65°F	
			2 ,,	67°F	
			5 ,,	67°F	
25–7–28	10	·0154	9 a.m.	58°F	Some embryos showing plu-
			12 p.m.	63°F	mules and radicles, others
			2 ,,	65°F	apparently undeveloped
			3·30 ,,	67°F	though swollen.
26–7–28	10	·0536	9 a.m.	58°F	Some radicles about $\frac{1}{4}$in. long,
			10·30 ,,	65°F	plumules short, no sign of
			3 p.m.	70°F	chlorophyll, some grains hardly started to germinate.
27–7–28	10	·0918	9·30 a.m.	64°F	Some radicles $\frac{1}{2}$in. long.
28–7–28	10	·0980	9·30 ,,	63°F	Experiment discontinued.
28–7–28	...	·0130	...		After boiling.

Total, CO_2 liberated, ·2718 gram.

One grain of *Booran* wheat therefore liberates 0.001359 gm. of CO_2 when germinated under the above conditions, the percentage CO_2 evolved being 2.913. At a solubility rate of 1,200 parts of CO_2 per million of water, the

CO_2 given off by one grain would be sufficient to saturate 1.1 gms. of soil solution.

The maximum solubility of the copper dusts used was found to be 224 parts per million in CO_2 saturated distilled water at 15°C. Assuming all the CO_2 evolved from 1 grain dissolved in the 1.1 gm. of soil solution, which is very unlikely, the maximum amount of copper dust that could be dissolved would be 0.000246 gm. Dusting at the rate of 2 ozs. per bushel (63 lbs.) the weight of powder retained on 1 grain is 0.0000924 gm., which could be easily dissolved by the CO_2 liberated. A germinating wheat grain will absorb its own weight of moisture up to the first appearance of chlorophyll. This weight represents .047 gm. of water which, when saturated with CO_2, could dissolve 0.0000096 gm. of copper dust. The position is then on excess of CO_2 and an insufficient supply of water for complete solution. If, however, the dissolved dust is absorbed by the spores, grain and soil, then the extra CO_2 will dissolve more dust and so keep up the concentration.

The copper dusts when examined microscopically reveal particles of different sizes. The smaller ones naturally dissolve first. In all solubility determinations excess of dust to solute has been taken, which means that the smaller particles have dissolved leaving the larger ones almost unattacked. Three samples, D25, E25 and B.S., were treated with an excess of H_2CO_3, i.e., an excess above their previously determined solubilities; after 18 hours standing with intermittent shaking each solution contained approximately equal quantities of copper, the coarse particles of each powder remaining undissolved. In D25 some finely divided material remained in suspension, this was basic carbonate contained in the original sample (the basic carbonates are less soluble in H_2CO_3 than the basic sulphates).

Further information, obtained at a later stage in this investigation, definitely shows, however, that the CO_2 theory has little bearing on the method of effecting solution of the copper compounds in the soil solution.

Solubility curves have been plotted showing the dissolved copper in soil extracts of different concentrations and uniform CO_2 content at a temperature of 30°C. The depressing action of the water soluble soil salts in Merredin loam is such that a lethal solution of copper due to CO_2 is never produced.
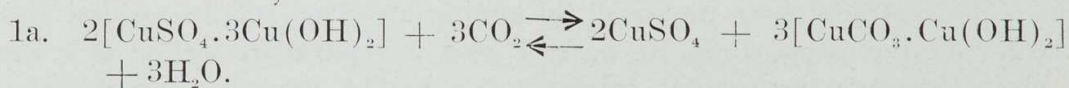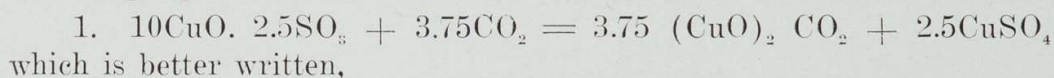
Soil extracts were prepared by adding air-dried soil to aerated distilled water in the proportions of 5, 2, 1 and .75 parts of water to 1 of soil. After thorough shaking at intervals for half a day the suspensions were allowed to stand overnight and filtered in the morning. Portions of each filtrate were evaporated to dryness and the dissolved salts determined. Over several days each of the filtrates was saturated with CO_2 and 25 ccs. withdrawn and added to .05 gm. of each of the dusts under test contained in a stoppered bottle. The bottles were shaken at intervals for several hours and rested on their sides overnight; the total time of contact was 18 hours and the temperature averaged 30-33°C. The suspensions were filtered in the mornings as rapidly as possible to avoid loss of CO_2, aliquots taken from the filtrates, and the dissolved copper determined by the ferrocyanide method. The curves on the graphs are not perhaps as even as they might be, but with warm weather prevailing the copper was more easily precipitated owing to the rapid loss of CO_2 on exposure of the solution to the atmosphere. Three samples only were filtered at the one time.

Graph No. 2 shows the rapid falling off in the solubility of the dusts with the increase in the concentration of the salts. Graph No. 3 shows the increase in concentration of extracted salts as the water added to the same quantity soil is decreased. The curve has been extended with cross lines and suggests when the soil contains about 10 per cent. moisture (field trials were planted and germinated well at Merredin at a moisture content of 10-12 per cent.) the salts not held by soil colloids, etc., would amount to 700 parts per million in solution. A continuation of the curves on graph 2 strongly indicates that at about 600 parts per million the copper dusts would be insoluble at temperatures of 30-33° C.

The maximum solubility of B.S. in H_2CO_3 is 112 parts per million of copper at 15°C. and at 30-33°C. 77.5 parts. Allowing for lower temperatures in the field at the time of planting the copper dissolved will not be sufficient for a lethal solution. E. E. Free has observed the falling off in the solubility of basic copper carbonate in H_2CO_3 when certain ions are present. The soluble salts in the Merredin loam (top 2in.) are normal for the soil type and are by no means excessive; they are, however, slightly higher in the sand. The soils are totally different. Graph No. 4 shows the variations in the soluble salts in the first two inches of Merredin loam, together with lines representing the mean soluble salts of both types. A sample was taken daily in 1928 for 20 days from different portions of the fungicide plots. The figures have been calculated to a dry soil basis. The original intention was to determine the solubilities of the dusts in soil extracts and see whether variations in the water soluble salts so affected the solution of the copper as to account for the variations in the counts in separate treatment rows.

The work of S. U. Pickering, C. T. Gimingham and B. T. P. Barker and C. T. Gimingham on the fungicidal actions of Bordeaux mixtures was only brought under the author's notice in December, 1931, after most of the experimental work had been done and definite conclusions drawn supporting almost entirely the views of Barker and Gimingham.

Pickering has studied the solubilities of various Bordeaux preparations in H_2CO_3 and made the observation that a dried out mixture is not as soluble as a freshly prepared one, which accounts for the low solubility of sample B.S. compared with some of his freshly prepared compounds. He gives the following equation:—

1. $10CuO. 2.5SO_3 + 3.75CO_2 = 3.75 (CuO)_2 CO_2 + 2.5CuSO_4$

which is better written,

1a. $2[CuSO_4.3Cu(OH)_2] + 3CO_2 \rightleftharpoons 2CuSO_4 + 3[CuCO_3.Cu(OH)_2] + 3H_2O.$

The reaction is reversible and would only proceed to completion on extreme dilution with H_2CO_3. The forward action depends on the CO_2 present. $CuSO_4$ reacts with $CuCO_3.Cu(OH)_2$: liberating CO_2. On passing CO_2 into a suspension of $CuSO_4.3Cu(OH)_2$ some Cu immediately passes into solution. The basic sulphate changes from a blue to a green colour in about 24 hours whether the CO_2 is passed continuously or the saturated suspension kept in a stoppered flask. On stopping the passage of the gas and exposing the suspension to the air, the dissolved Cu and SO_4 are precipitated in a few hours while the suspended material remains green. A little CO_2 is permanently absorbed by the basic sulphate.

Returning to the germinating grain of wheat in the moist seed bed. The ratio of water absorbed to the copper dust is $\frac{.047}{.0000924}$, i.e., approximately 500 to 1. The solubility tests were carried out at the ratio 25/.05,

i.e., 500 to 1. At this proportion 224 parts of B.S. (50 per cent. Cu content) were soluble in one million parts of H_2CO_3 at 15° C. Neglecting any Cu dissolved as carbonate in the above equation the extent to which the forward reaction has taken place can be calculated.

At a 1-500 dilution the actual Cu dissolved from .05 gm. of B.S. is 0.0028 gm., *i.e.*, 11.2 per cent. of the copper contained in it on the assumption that B.S. contains 50 per cent. Cu (actually the percentage is 52). At a 1-500 dilution the reaction will therefore reach equilibrium when 11.2 per cent. of the copper in the basic sulphate (containing 55 per cent. Cu) has dissolved, *i.e.*, 45 per cent of its contained $CuSO_4$ has been liberated. At this dilution the reaction has proceeded 45 per cent. of its way. This conclusion supports Pickering's contention that the dried out dusts are less soluble. Except with extreme dilution samples D25, E25 and B.S. will not dissolve completely, due to the formation of the very insoluble basic carbonate. Pickering again shows theoretically that Bordeaux mixtures prepared with different proportions of lime give basic sulphates of different composition. He argues that $10CuO,SO_3$ would give less soluble copper than $4CuO,SO_3$ and correctly so, but on treatment with H_2CO_3 more copper appears in solution than is expected. This can only be due to the solubility of the basic carbonate formed. Once the liquid spray has dried out its solubility falls, undoubtedly due to change in particle size.

Free when investigating the solubility of freshly prepared basic carbonates found their initial solubilities in H_2CO_3 varied from 57 to 107 parts per million, but by treating a suspension of the material with the gas for several days he prepared a product which gave a solubility of approximately 35 parts per million of Cu in water saturated with CO_2. He found that the addition of certain substances to the solvent affected the solubility of the basic carbonate, *e.g.*, sodium chloride increased the solubility slightly while sodium carbonate and calcium carbonate decreased it. C. A. Seyler's explanation of this phenomenon is a satisfactory one—the basic carbonate dissolved as bicarbonate.

$$CuCO_3.Cu(OH)_2 + 3H_2CO_3 \dashrightarrow 2H_2O + 2Cu(HCO_3)_2$$
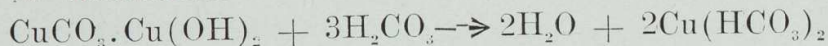
and at equilibrium

$$\frac{[Cu]^2\,[HCO_3]^4}{H_2CO_3} = K$$

The addition of Cu or HCO_3 ions will therefore decrease the solubility of the Cu. This explanation may be applied to the depression of the solubility of the copper dusts in the soil solution which contains the ions Cl, HCO_3, SO_4 and NO_3 only in quantity. It can be definitely stated that Pickering's contention that the copper in sprays is rendered sufficiently soluble by CO_2 to be toxic to fungi on plants is untenable when applied to copper dusting powders on wheat grains germinating in the soil. The hydrogen ion concentration of the soil solution, too, would have an important bearing on the solubilities of the dusts.

Dismissing the CO_2 solubility theory the only other explanations for the solubility are as follows:—

1. Solution in a secretion of the spores.
2. Decomposition products of the germinating grain other than carbon dioxide.
3. Contact with injured portions of the grain.

D. DETERMINATION OF SPECIFIC CHEMICAL AND PHYSICAL CHARACTERISTICS.

Mackie and Briggs suggested the apparent density for differentiating between copper dusts of uniform chemical compositions but different fungicidal properties. Unfortunately their work is based on only four samples tried out in the field for one season. Their standards have been arrived at by averaging the analyses of twelve basic carbonates selected from a series of twenty samples. Some of the densities of the selected twelve are given and of these, two only reach the required density. Experience in Western Australia and Victoria shows that some high density compounds are better than low density ones. In view of this fact it is highly probable that had Mackie and Briggs tried out all their samples in the field they would have looked for some other property in their dusts which could have been associated with bunt control. Apparent density cannot be correlated with particle size unless the particles are themselves uniform in size—the small particles will fill the spaces between the larger ones and so increase the density.

A rock sample was broken in a crusher and the material sifted through a series of I.M.M. sieves. The apparent densities of the fractions were determined with and without tamping. Quantities of material were added at intervals to a 50 ml. cylinder and tamped down on a wooden bench covered with a cloth; the maximum amount of material that would occupy 30 mls. volume was weighed. The other densities were determined by pouring a quantity of material into a 2½in. funnel without a stem and supported one-half an inch above a cut-down cylinder of 30 mls. capacity. The uneven surface was struck off and the contents weighed. Good duplicate results were obtained.

Apparent Density of Samples.

Mesh.	Without tamping.	With tamping.
20–30	1·220	1·403
30–60	1·140	1·377
40–120	1·062	1·327
120–200	1·008	1·298
<200	0·724	1·256

It is clear from these figures that with particles of uniform size the apparent density decreased with a decrease in particle size.

Increasing amounts of the finest material were then added to the coarsest material and the apparent densities of the mixtures determined.

APPARENT DENSITIES OF MIXED MATERIAL.

Proportions of coarse to fine material by weight.							Without tamping.	With tamping.
Coarse	<200							
100	20	...	...	...	...	...	1·232	1·572
100	40	...	...	...	...	...	1·233	1·680
100	60	...	...	...	...	...	1·144	1·708
100	80	...	...	...	...	...	1·109	1·696

The specific gravities of the coarse and fine materials were 2.53 and 2.60 respectively. Without tamping the "lightening" effect of the fine particles

on the apparent density of the coarse ones does not make itself felt until the mixture contains about 30 per cent. of fine particles; below this figure the value is increased. With tamping the maximum apparent density is obtained when the mixture contains about 40 per cent. of fine material; the value then begins to fall. There is no reason why this phenomenon should not make itself apparent in mixtures of copper dusting powders in which the particles may range in size from 200 mesh to a 1,000 mesh or smaller. It is possible that to get comparative results the amount of tamping should be governed by the mechanical composition of the powders.

Mackie and Briggs determined densities "by shaking down a given weight in a measured cylinder," neither quantities nor the amount of shaking being specified. Chemists have interpreted these instructions according to their own ideas. The densities in this report have been determined by tamping down one ounce of material in a 50 ml. cylinder until the volume is constant. Twentyman has evolved a satisfactory method in which the conditions have been standardised.

Table 6 gives the results obtained with three samples of basic carbonate of varying densities during the year 1928 only.

TABLE 6.

Spore dosage 10–750—Variety of wheat *Booran*.

| Sample | | | B 25 | | B 27 | | A B | |
Density			58·7		53·8		49·0	
			Loam.	Sand.	Loam.	Sand.	Loam.	Sand.
Per cent. bunted plants			33	3	51	7	57	12
Per cent. infection	controls		83	91	83	91	83	91

It will be seen that those samples recording high densities are better in practice than the low densities under the conditions of these experiments. The evidence is not complete and it is not suggested that other basic carbonates would behave in the same way. In fact other investigators show that light powders are better than heavy ones. The inference is that density determinations do not necessarily divide good from bad commercal powders.

Difficulty arises in distinguishing between those powders which contain varying proportions of basic carbonate and basic sulphate, as the true specific gravities differ. The oxychlorides complicate the position still further. Twentyman's figures when graphed do not show uniformity in relation to bunt control; the graph for all samples fluctuates, while separate graphs of the basic carbonates, basic sulphates and mixtures follow the same form.

While it is admitted apparent densities are a measure of fineness when the particle size is uniform or particles of similar size predominate, the fact remains that only in some cases do densities foretell how powders will behave in field trials.

Sample AB was supplied by the manufacturers and was stated by them to have an apparent density of 30.5 lbs. per cubic foot. A known weight of this powder was tamped down in a 50 ml. cylinder until a volume was obtained giving a density value of 30.5 lbs. per cubic foot. The same quantity of B25 was tamped under exactly the same conditions and gave a value of 34.1. B25 has given better results in the field than AB. By tamping

to a minimum volume, *i.e.*, maximum apparent density, the figures were:—
AB 49 and B25 58.7. Twentyman obtained a value 33.5 for the brand AB
by his method but whether the two samples were similar is of course un-
known. However repeating the above determinations by tamping AB to
33.5 the B25 figure became 39.9. Twentyman has been good enough to
determine the density of sample A27 for the author and obtained the figure
54 lbs. per cubic foot; he points out that the result is a close approximation
as only an ounce of material was sent to him. A27 tamped right down went
89 lbs. As will be seen from Tables 2 and 3 this material gave good results in
trials. Whether densities are determined with controlled or maximum tamp-
ing a relationship exists between the figures obtained, *i.e.*, the West Aus-
tralian figures may be compared with those of other investigators.

FINENESS.

The results of field trials particularly with the basic sulphates suggest
that the powders can be too fine. Sample BS is a very coarse dust while E25
is very fine, the former is by far the better bunticide, and has a lower
apparent density. The low apparent density is ascribed to the scarcity of
small particles to fill the spaces between the larger ones. Again the differ-
ence may be due to the different chemical compositions or the hardness of
the particles. Some coarse particles in both dusts, particularly B.S., have
a spongy appearance and may present a greater surface to solvents exuded
by the bunt spores.

In 1929 an attempt was made to separate the finest fraction from the
coarse ones with a stream of air. The apparatus was not designed for quan-
titative determinations. Coarse sand was added to the powders to facilitate
agitation under constant air pressure. The fine and coarse materals were
used in field trials together with sample B.S.

TABLE 7.

Rate of infection 10–750, *Gluyas Early* wheat. Planted in Merredin loam.

					Per cent. bunted plants.	
					1929.	1931.
Average controls ...	...	...	...	...	41·6	8·6
B25 original	...	...	...	...	12·4	0·0
B25 fine ...	...	...	...	...	9·4	1·7
B25 coarse	...	...	...	...	7·2	1·2
D25 original	...	...	...	...	4·4	0·9
D25 fine ...	...	...	...	...	5·4	0·9
D25 coarse	...	...	...	...	10·2	0·3
AB fine ...	...	...	...	...	5·2	2·1
AB coarse ...	...	...	...	...	4·0	1·5
B.S. original	...	...	...	...	2·2	0·7

Booran wheat—
　　Infected 10–750　　...　　...　　...　　...　　67·0

The 1929 trials did not give the results expected, in fact no significant
information can be gathered from them, while the 1931 figures complicate
the position still further. Planting in 1931 was completed on 11th June and
was followed by dry conditions with extremely low temperatures. Sixteen
frosts were recorded during the remainder of the month, on one occasion the
terrestrial minimum was as low as 20.7° F. The low bunt infection asso-
ciated with cold conditions and slow germination of the wheat is a point of

significance when comparing one season's results with others. In 1929 the coarse and fine fractions of AB gave better results than those of B25 and *vice versa* in 1931. D25 and B.S. still maintain their superiority.

The use of the 200-mesh sieve as a test for fineness is the subject of much controversy. It is the finest sieve in general use in most laboratories and appears to be a good one to separate ineffective lumps from fine material and insure at least a dust is supplied to farmers.

EFFECT OF CARBON DIOXIDE ON THE MOISTENED DUSTS.

Weighed quantities of six of the dusts were spread out on moistened filter papers and placed in an atmosphere of moist carbon for eight days. The papers were allowed to air dry and the absorbed carbon dioxide determined. The increases were as follows:—

Sample	AB.	B25.	F25.	D25.	B.S.	E25.
Absorbed CO_2 per cent.	0.28	2.36	0.65	0.40	3.54	0.84

Sample D25 had a distinctly blue colour while B.S. was muddy blue. All samples turned to a bright green after a few days contact with the carbon dioxide and retained their new colours on drying.

COLOUR.

The majority of the commercial copper dusting powders are green in colour. Sample D25 which has given consistently good results has a distinctly bluish green colour. It would appear that colour has no relationship to fungicidal properties.

SUMMARY.

Field trials have been conducted with commercial "copper carbonates" over a period of years in different soils with a view to fixing standards for these materials.

Beyond the fact that the compounds should contain approximately 50 per cent. of copper and be reasonably fine, no constant physical or chemical characteristic could be found which would distinguish a good powder from a bad one. Generally speaking basic copper sulphates and mixtures of basic sulphates and carbonates have proved better bunticides than the basic carbonates or oxychlorides tested.

The Merredin loam soil solution depresses the solubility of the copper powders in carbonic acid derived from the germinating grain to below a lethal dose.
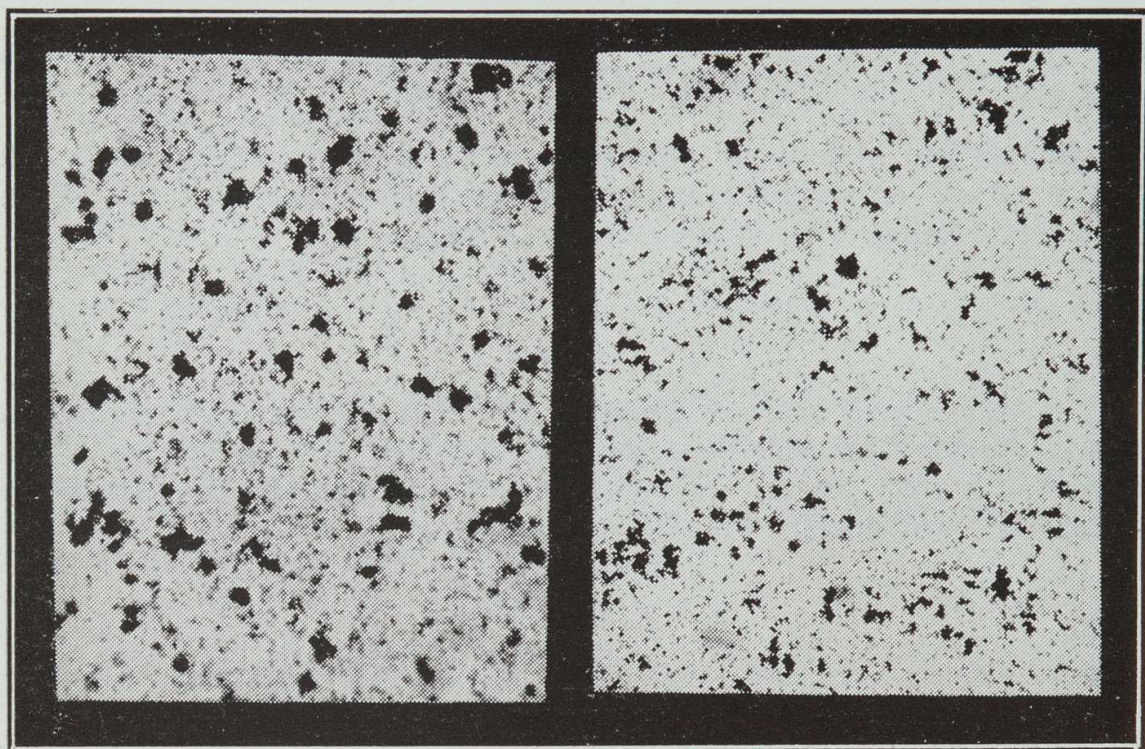
The amount of powder actually held on the dusted grain is not a measure of the efficiency of the powder.

The apparent density test is not a reliable one for indicating bunticidal properties.

STANDARDS.

The following standards are recommended for commercal "copper carbonate" powders:—

(a) They shall contain not less than 50 per cent. of copper in the form of basic carbonate, basic sulphate, mixtures of these compounds, or oxychlorides.

(b) 98 per cent. of each powder should pass through a 200-mesh I.M.M. sieve when shaken with water and washed with a gentle stream of water.

(c) Water soluble copper should not be present.

B S.   B 25.

Glycerine dispersion.   × 70.

Microphotographs of dusting powders.

ACKNOWLEDGMENTS.

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